kidneys using two-photon microscopy. Fibrillar collagen was detected surrounding interlobar vascular bundles in embryonic day 17 mouse kidneys using second harmonic generation, a non-linear optical process. Additional studies are underway to examine endothelial–matrix interactions in E12–E13 kidneys using SHG and 2-photon microscopy in both fixed and living embryos.

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Program/Abstract # 345
Prox1 is a critical regulator of pancreatic development and homeostasis
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Our previous studies revealed that the function of the divergent homeodomain transcription factor Prox1 is required to control multiple aspects of early pancreas organogenesis (e.g., epithelial growth, branching morphogenesis, exocrine cell differentiation and timely islet cell genesis). Using a conditional knock-out approach recently we showed that the loss of pancreatic Prox1 function does not compromise mouse viability, but it severely affects the differentiation of pancreatic exocrine and ductal cells and it gradually impairs islet cell physiology. Consequently, these mice exhibit a massive loss of exocrine tissue, they become glucose intolerant and develop ductal abnormalities similar to those observed in patients with pancreatitis. In addition, Prox1 conditional knock-out newborn pups and young adults also exhibit abnormal, continuous production of islet cells (islet neogenesis), a phenotype suggesting that specific regenerative responses are activated in these mutant tissues after birth. Thus, mice lacking pancreatic Prox1 function provide us with a valuable animal model to study both, specific aspects of pancreas development and homeostatic responses in the postnatal pancreas.

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Program/Abstract # 346
Conditional control of pancreatic progenitor maintenance and differentiation by FGF10 uncovers an endocrine-specific competence window in development
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FGF10 is a critical component in mesenchymal-to-epithelial signaling during endodermal development. In Fgf10 null pancreas, the embryonic progenitor population fails to expand whereas ectopic FGF10 expression during pancreagenesis force progenitor arrest and hyperplasia. Using a conditional Fgf10 gain-of-function model, we find that the arrested progenitor state is reversible, and that terminal differentiation may resume upon cessation of FGF10 production. However, the competence towards individual pancreatic fates is dependent on gestational time, revealing a temporal window where endocrine cells may form coinciding with the pancreatic secondary transition occurring at E13.5–E15.5. We demonstrate that maintenance of arrested state beyond this timepoint leads to an irreversible loss of the endocrine, but not exocrine lineage competence.

Program/Abstract # 347
Function of Ctgf in islet development and beta cell proliferation
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The individual factors necessary for normal pancreatic islet morphogenesis are not well characterized. We have found that connective tissue growth factor (CTGF) is involved in islet morphogenesis and embryonic β cell proliferation. CTGF is a secreted protein that modulates several growth factor signaling pathways including TGF-β, BMP, and Wnt. Although CTGF is essential for normal skeletal development, its role in pancreas development has not before been examined. Using a LacZ-tagged CTGF allele, we show that CTGF is expressed highly in pancreatic ductal epithelium and at lower levels in developing endocrine cells, but becomes down-regulated in β cells soon after birth. Pancreata from embryos lacking CTGF have increased glucagon+ cell area and show defects in islet organization and migration away from the ductal epithelium. Loss of CTGF also results in a dramatic decrease in β cell proliferation at late gestation. CTGF is re-expressed in maternal islets during pregnancy, suggesting that it may play a role in β cell proliferation under physiologically stimulatory conditions. To determine the cellular source of CTGF in the developing pancreas that is required for β cell proliferation and islet morphogenesis, we have generated a conditional allele of CTGF to allow for cell type-specific gene inactivation. Using Cre-lox technology, we will examine
whether CTGF is required in the pancreatic endodermal epithelium, vasculature, or β cells themselves.

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Program/Abstract # 348
Cross-talk between neural crest cells and developing pancreatic epithelium regulates beta-cell mass
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In the ventral hindbrain, mouse homeodomain transcription factor Phox2b works in concert with Nkx2.2 to determine subtype identity of neurons. In the periphery, Phox2b is expressed by neural precursors that colonize the gut to form the enteric nervous system. We found Phox2b briefly expressed in E12.5 mouse embryonic pancreas, in cell nuclei along the epithelial–mesenchymal border, and down-regulated in an Nkx2.2-dependent manner shortly afterwards. Lineage tracing with β-galactosidase staining of Pdx1-Cre/Rosa26-LacZ embryos indicated that Phox2b-expressing cells did not originate from Pdx1-expressing pancreatic epithelium. Instead, the timing of pancreatic Phox2b expression, its mesenchymal localization and co-localization with SOX10 are all consistent with its expression in neural-crest derived cells in other parts of the gut tube. In addition, Pgp9.5-positive differentiated neurons were absent from both the pancreas and the stomach of mice lacking functional Phox2b alleles. On the other hand, its transient expression and Nkx2.2-dependency represent unique aspects of pancreatic Phox2b expression. Interestingly, the pancreas of Phox2bLacZ/LacZ transgenic mice contained increased numbers and replication of insulin-expressing cells, as well as increased Nkx2.2 expression. We conclude that, during pancreatic development, Phox2b and Nkx2.2 form a non-cell autonomous negative feedback loop that connects the neural crest to pancreatic epithelium. These data represent the first evidence that innervating neurons regulate beta-cell mass.

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Program/Abstract # 349
ptf1a determines pancreatic exocrine versus endocrine fates
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Understanding how progenitor cells of the pancreas are specified to differentiate into endocrine cells has profound implications on diabetes therapeutics. Although the bHLH encoding gene, ptf1a, is required for exocrine differentiation, ptf1a is expressed in the progenitor cells of all pancreatic cell types. To determine the function of ptf1a in endocrine cell fate specification, we examined the differentiation of ptf1a mutant cells using a zebrafish ptf1a mutant in the transgenic ptf1a:GFP reporter background. In contrast to previous conclusions, we find that endocrine cell neogenesis occurs independent of ptf1a function. Furthermore, we show that cells that would normally express ptf1a and become exocrine cells, are transdetermined towards an endocrine fate in ptf1a mutants, indicating that ptf1a represses endocrine differentiation. This transdetermination of ptf1a expressing cells is not observed in other embryos with defective exocrine differentiation suggesting that ptf1a plays a specific and crucial role in determining exocrine versus endocrine specification. We conclude that exocrine and endocrine cell fates are not predetermined, and that the down-regulation of Ptf1a function is required to allow for endocrine cell specification. This finding provides a potential mechanism for exocrine to endocrine cell transdifferentiation, which has substantial implications on pancreatic endocrine cell replacement therapy for diabetic patients.

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Program/Abstract # 350
Dkk-1 and Nodal function in parallel to induce both heart and endodermal organs such as liver and pancreas
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We recently demonstrated that two genetic pathways, one mediated by antagonists of canonical Wnt/b-catenin signaling, the other activated by Nodal signaling, function in parallel to pattern the early heart field. Wnt antagonists such as Dkk-1, Gsk3b and a dominant negative form of the transcription factor TCF3 pattern the heart indirectly by inducing expression of the transcription factor Hex in the endoderm underlying the presumptive heart field, whereas the Nodal homologue (XNr-1) functions by inducing the secreted molecule Cerberus. Since both pathways function in the endoderm fated to become liver and pancreas, we asked (1) whether these pathways influence differentiation of endodermal organs, and (2) the identity of the secreted factor(s) induced by Hex or Cerberus that induce heart tissue. Q-RTPCR and in situ hybridization analyses show that both Wnt/b-caten antagonists and XNr-1 re-pattern endoderm to express markers for differentiated endoderm, including liver and pancreas. Interestingly, cell autonomous and cell non-autonomous effects of Wnt/b-catenin signaling led to the induction of distinct endodermal derivatives. Moreover, in the case of Dkk-1, this tissue is capable of forming insulin-positive islet cells. Finally, we have identified several potential downstream factors by screening two Xenopus gene micro-arrays. The candidate inducing proteins are being testing for...